

On the taxonomy of the entomogenous fungus *Filobasidiella arachnophila*

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The taxonomic status of *Filobasidiella arachnophila* Malloch et al. was investigated. The carbohydrate profile of two strains revealed basidiomycetous affinities. However, the vast majority of the mycelial cells are monokaryotic, demonstrating that *F. arachnophila* is not a typical basidiomycete. The morphological resemblance to the two teleomorph species of *Filobasidiella* is noteworthy and therefore the accommodation in *Filobasidiella* is maintained. *F. arachnophila* proved to be identical with *Aspergillus depauperatus* Petch and the new combination *Filobasidiella arachnophila* (Petch) Samson et al. is made.

INTRODUCTION

In the course of taxonomic studies on entomogenous fungi the type specimen of *Aspergillus depauperatus* Petch was examined. This fungus was described by Petch (1931) on scale insects and has not been reported since. However, recently Malloch et al. (1978) found a similar fungus on a dead spider in Canada and described it as *Filobasidiella arachnophila*. The species was considered homothallic and differed from the other two species of the basidiomycetous yeast genus *Filobasidiella* (Kwon-Chung, 1975, 1976 a, b) by the different spore shape and the absence of a yeast phase and clamp connections. These characters would suggest that the accommodation in *Filobasidiella* is not appropriate, and that the basidiomycetous affinities may be questioned. However, Khan et al. (1981) found that the septal ultrastructure of *F. arachnophila* is similar to that of other basidiomycetes and typical of the Filobasidiaceae. These authors did not establish the generic position, because they could not determine whether the spores were mito- or meiospores.

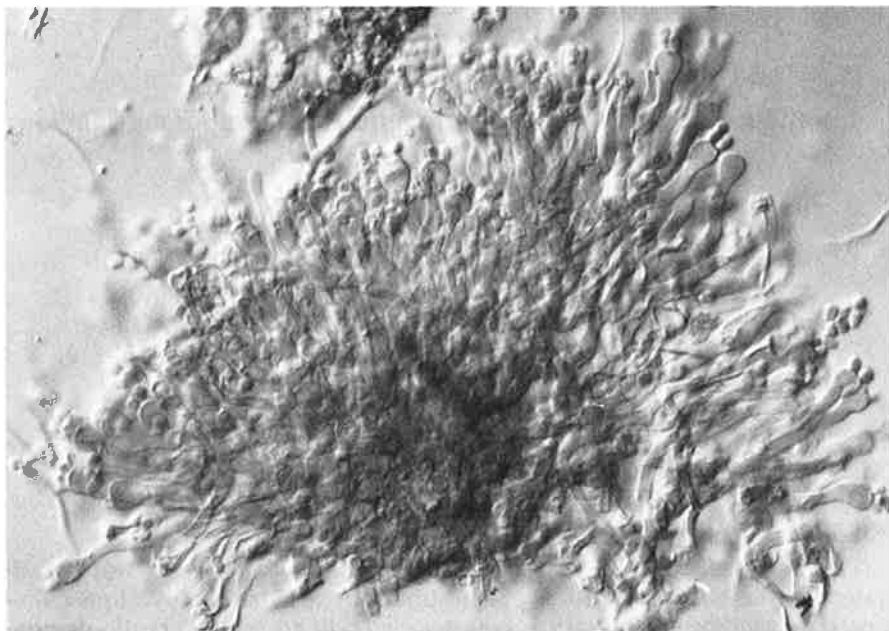


Fig. 1. Nomarski interference microscopy of the sporophores of *Aspergillus depauperatus* (holotype) arranged in a sporodochial manner ( $\times 700$ ).

In this paper the morphological characters and the carbohydrate cell wall composition of *F. arachnophila* are compared with those of the type species of *Filobasidiella*, *F. neoformans*. The nuclear behaviour was examined using fluorescence microscopy. Furthermore the identity of *F. arachnophila* with *A. depauperatus* is established.

#### MATERIALS AND METHODS

*Aspergillus depauperatus*, holotype R 535, on *Aspidotus* sp., collected in Nuwara Eliya, Sri Lanka, 31.8.1928 by T. Petch (in herb. K).

CBS 511.80, isolated from aphids together with *Verticillium lecanii* on leaves of *Raphanus sativus*, Wageningen, The Netherlands.

*Filobasidiella arachnophila*, holotype TRTC 48044 on dead spider, Mississauga, Ontario, Canada, leg. D. Malloch 30.10.1976; culture B 3810 derived from the holotype of *F. arachnophila*, obtained by courtesy of K. J. Kwon-Chung.

For scanning electron microscopy the cultures were grown on Sabouraud-glucose agar and prepared according to the technique described elsewhere (Samson et al., 1979).

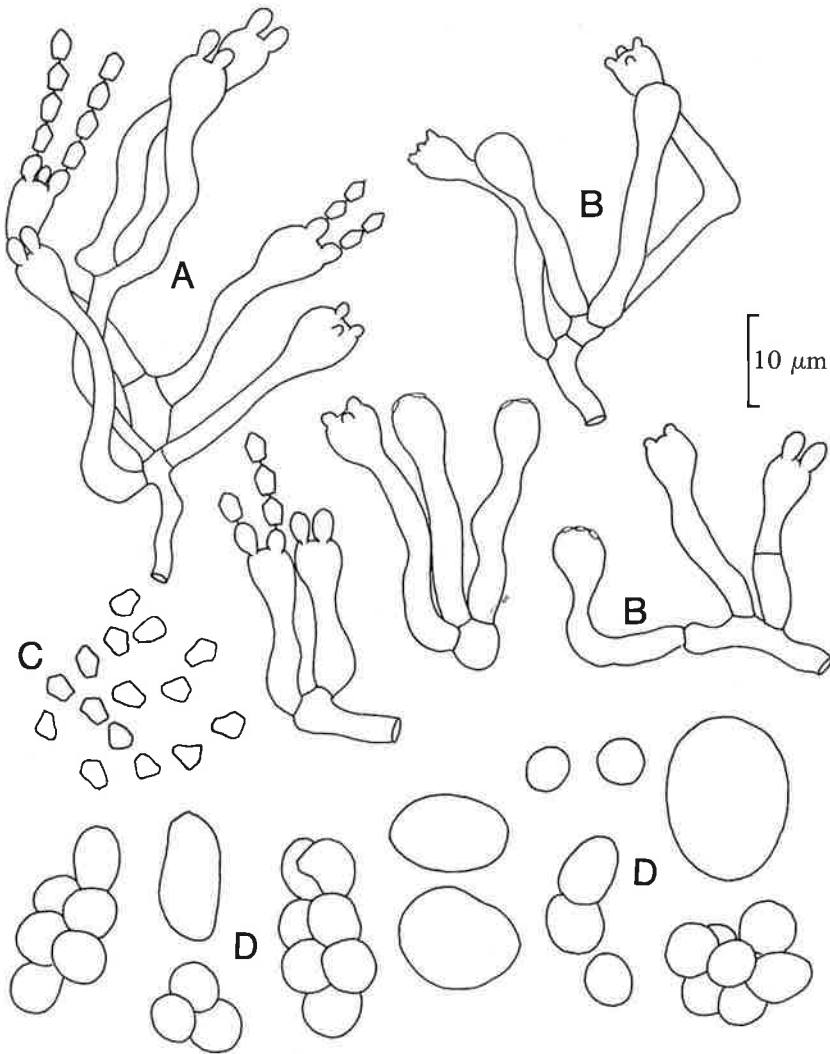


Fig. 2. Camera lucida drawings of the sporogenous structures of *Aspergillus depauperatus*. A, B, sporophores, C, spores, D, hyphal bodies inside scale insect.

To examine the nuclear characteristics the nuclei were either stained with acridin orange in distilled water (1:1000) and observed with filter I2 or stained in a 1:1 mixture of (a) 5 mg mitramycin and 125 mg  $MgCl_2$  in 100 ml 12.5% ethanol and (b) 2.5 mg ethidium bromide, 1.2 g Tris buffer, 0.6 g NaCl in 100 ml water (pH 7.4) and observed with filters G and I2 (Barlogie et al., 1976). They were investigated with a Leitz Dialux microscope with Ploemopak fluorescence device.

The strains were grown on glucose (2%) – peptone (1%) – yeast extract (0.5%) medium in infusion bottles for 10 days at 25 °C under continuous shaking at 150 rpm and prepared for carbohydrate analysis according to Weijman (1978).

For GLC analysis of sugars and polyols, freeze-dried whole cells were chromatographed as their trimethylsilyl derivatives according to Sweeley et al. (1963), after hydrolysis in 1N HCl.

Analyses were performed using a Hewlett-Packard HP 5880A gas chromatograph and a HP 5993B gas chromatograph-mass spectrometer-data system equipped with fused silica open tubular capillary columns (length 25 m, i.d. 0.32 mm, coated with OV-101). A more detailed description is given by Weijman et al. (1982).

## RESULTS

### *Taxonomy*

*A. depauperatus* was reported by Petch (1931) as rather abundant on *Lepidosaphes ulmi* on hawthorn in Norfolk, England in 1921. However, the species was first described after Petch collected it again in 1928 on an *Aspidotus* species in Sri Lanka. The description was based mainly on this collection, which is in excellent condition in herbarium K. The specimen contains several leaves with numerous scale insects. Not all of the scales are infected by fungi, but many of them are covered by minute white patches of fungal mycelium. Some of these scales are parasitized by *V. lecanii* (Zimm.) Viegas, but this species is not abundant. The white patches are clusters of sporogenous structures of *Filobasidiella*, mostly densely packed in a sporodochial manner (Fig. 1). The branched sporogenous structures arise directly from the cuticle (Fig. 2). Vegetative mycelium is very scarce, the hyphae are smooth-walled, hyaline and 2.0–2.5 µm wide. Inside the body of the scale subglobose to ellipsoidal hyaline cells (5–17 µm diam.) were observed, often occurring in clusters (Fig. 2 D). The sporogenesis of these propagules could not be determined in the herbarium specimen.

The recent collection on aphids from *Raphanus* leaves revealed insects heavily infested by *V. lecanii*. The sporogenous structures of *A. depauperatus* occurred on or between the hyphae of *V. lecanii*. The bodies of the aphids were filled with various kinds of cells, originating from both *V. lecanii* and *F. arachnophila*. Microscopical comparison of both collections of *A. depauperatus* with the holotype of *F. arachnophila* showed that they were identical.

Fig. 3A, B again demonstrates the presence of clamp connections and the *Cryptococcus* anamorph in the type culture of *F. neoformans*. In both cultures of *F. arachnophila* these structures are absent. The morphological similarity of young sporogenous structures of *F. neoformans* and *F. arachnophila* is striking (Fig. 3C, D). The micrographs show the four sporogenous loci at the swollen

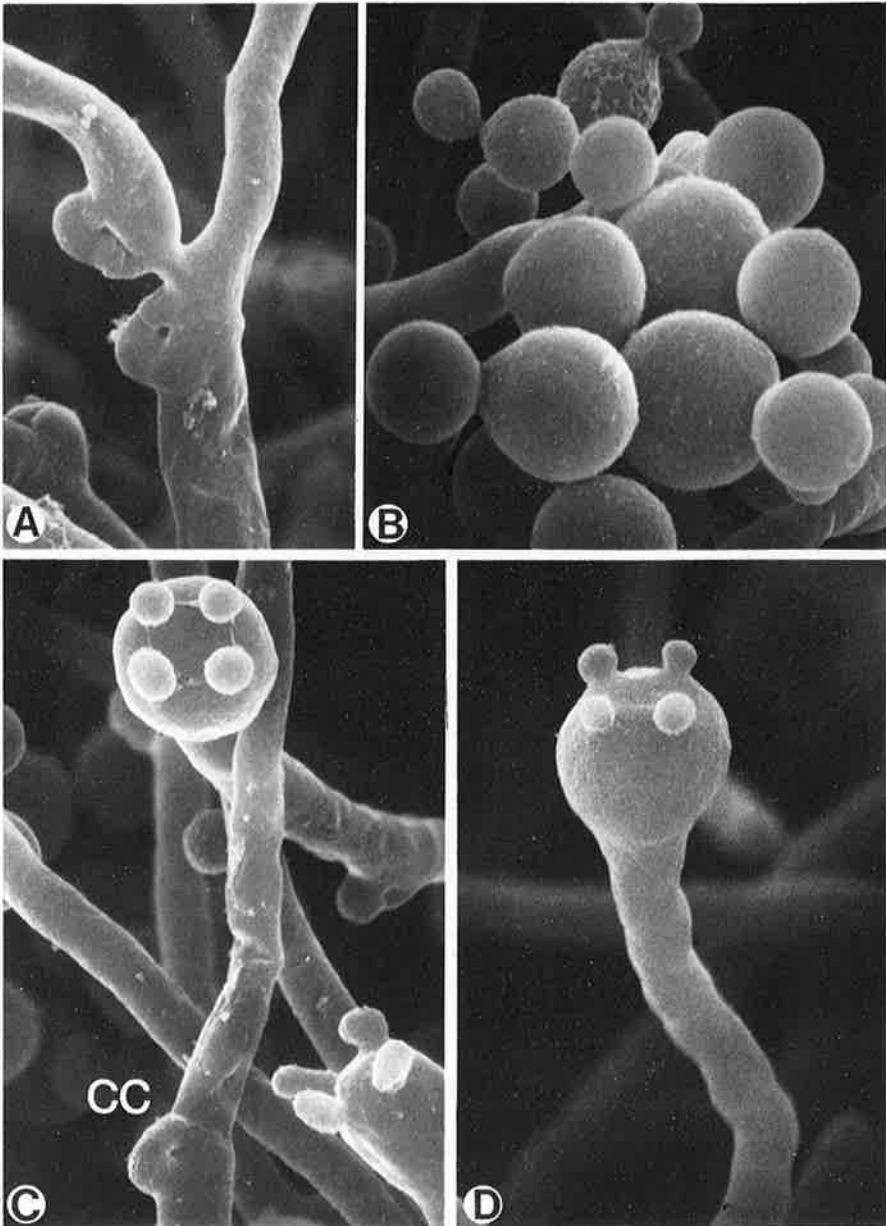


Fig. 3. Scanning electron micrographs of *Filobasidiella neoformans* A. clamp connections ( $\times 4700$ ), B. *Cryptococcus* anamorph ( $\times 4700$ ), C. young basidium. Note the clamp connection (cc) ( $\times 4000$ ), D. *F. arachnophila*, young sporophore ( $\times 7000$ ).

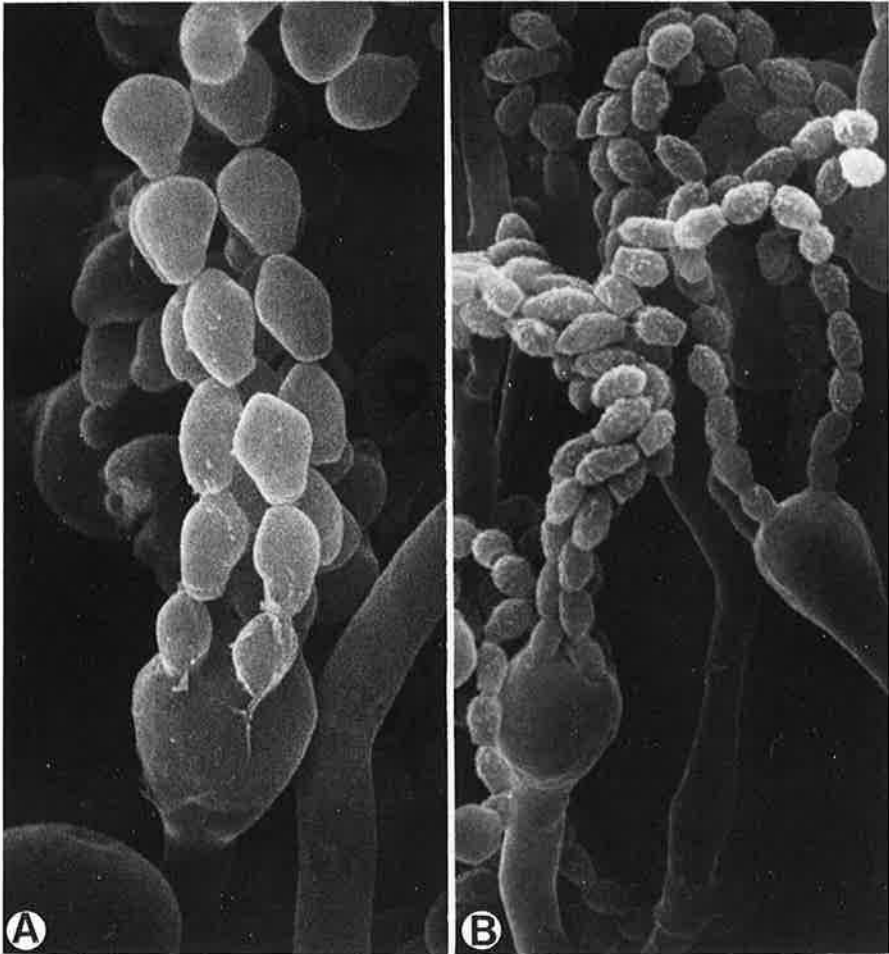


Fig. 4. Scanning electron micrographs of mature sporogenous structures of *Filobasidiella arachnophila* (A) and *F. neoformans* (B), showing the four basipetal chains of spores, A.  $\times 6000$ , B.  $\times 3500$ .

apex. The mature sporophores of both species (Fig. 4A, B) are also very similar. Sporophores of both species give rise to four basipetal chains of truncate spores. In *F. arachnophila* the spores are smooth-walled, obpyriform or oblong-pentagonal and measure  $2.8\text{--}4.0 \times 2.2\text{--}2.9 \mu\text{m}$ , while in *F. neoformans* they are rough-walled, ellipsoidal to cylindrical and  $1.8\text{--}2.5 \mu\text{m}$  in diameter.

#### *Nuclear characteristics*

The vast majority of the mycelial cells are monokaryotic. In the sporogenous

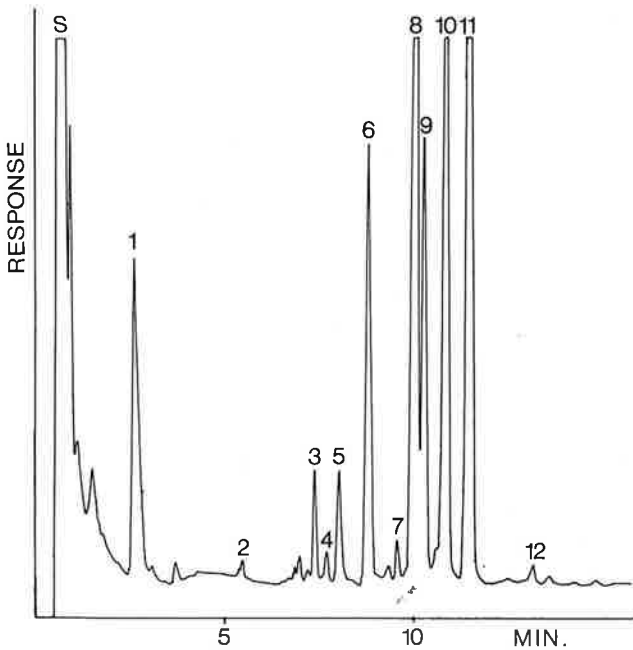


Fig. 5. Gas chromatogram of carbohydrates (TMS-derivatives) released from whole cells of *Filobasidiella arachnophila* strain CBS 511.80 by hydrolysis in 1N HCl. S solvent; 1 glycerol; 2 tetritol (probably erythritol); 3  $\alpha$ -xylose; 4 pentitol (probably arabitol); 5  $\beta$ -xylose; 6  $\alpha$ -mannose; 7  $\alpha$ -galactose; 8  $\alpha$ -glucose; 9  $\beta$ -mannose and  $\beta$ -galactose; 10 hexitol (probably mannitol); 11  $\beta$ -glucose; 12 myo-inositol.

cells up to four nuclei were observed; at a certain stage these can be found near the sporogenous loci and inside the developing spores. The spores are monokaryotic. Mating of monospore isolates of both cultures neither revealed binucleate cells nor clamp connections.

#### *Carbohydrate profiles*

The cell hydrolysates contain mainly glucose, mannose and some xylose. The carbohydrate pattern of the *Filobasidiella depauperata* strains (CBS 511.80 and B 3810), as studied by GLC and GC-MS of whole cell hydrolysates (Fig. 5), closely resembles the pattern of *Filobasidiella neoformans* (Von Arx and Weijman, 1979) and other basidiomycetous fungi, e.g. *Filobasidium*, *Bullera*, *Trichosporon*, *Cryptococcus*, *Sporotrichum*. In all these fungi glucose is dominant in the hydrolysates, whereas mannose is less abundant. The presence of xylose is a characteristic indication of basidiomycete affinity (Von Arx and Weijman, 1979; Weijman, 1979).

## DISCUSSION

The few reports of *F. depauperata* on scales, aphids and a spider suggest a wide host range among the arthropods. It seems, however, that this fungus occurs mainly in the presence of other entomopathogenic fungi, e.g. *V. lecanii*, as observed by Petch (1931), Malloch et al. (1978) and the present authors. This may indicate hyperparasitism on other entomogenous fungi rather than pathogenicity of the arthropod host.

Our carbohydrate data confirm the conclusion of Khan et al. (1981) that *F. arachnophila* has basidiomycetous affinities.

The nuclear data can be interpreted in two different ways: the fungus is either a haploid or a diploid basidiomycete. The first possibility was mentioned by Khan et al. (1981). In haploid basidiomycetes basidia are formed which resemble normal basidia in all respects except that no sexual processes take place. The spores are then mitospores. The phenomenon, which is known as haploid fruiting, is well-known in higher basidiomycetes such as *Schizophyllum commune* Fr.: Fr. (Wakefield, 1909; Esser et al., 1979), *Athelia rolfsii* (Curzi) Tu & Kimbrough (Punja et al., 1982) and *Flammulina velutipes* (Curt.: Fr.) Sing. (Stalpers, unpublished observation). For more examples see Prillinger (1982).

Erke (1976), Kwon-Chung (1978) and Schmeding et al. (1981) reported strains of *F. neoformans* with monokaryotic hyphae, incomplete clamp connections and basidia with basipetal chains of spores. Such self-fertile strains could produce self-sterile colonies which were bipolar. Kwon-Chung (1978) suggested that the self-fertile strains of *F. neoformans* were uninucleate and diploid. Partly diploid mycelia are known for example in *Armillaria mellea* (Vahl.: Fr.) Kummer (Korhonen and Hintikka, 1974; Ullrich and Anderson, 1978). This may also be possible for *F. arachnophila* in which case the basidiospores would be meiospores.

Further genetic studies are required to determine which of these possibilities applies to *F. arachnophila* and thus whether the spores are sexual or not. If *F. arachnophila* is asexual and thus an anamorph, it cannot be placed in *Filobasidiella* (Weresub and Hennebert, 1979). Similar situations in the higher basidiomycetes do exist, as e.g. for *Clavulinopsis* (Petersen, 1978), but generally the sexual state is known and named, so that there is no need for an anamorph name. For the moment the striking resemblance of *F. arachnophila* and *F. neoformans* and the probable occurrence of diploid strains in the latter species justify the inclusion of *F. arachnophila* in *Filobasidiella*.

Our taxonomic studies revealed that *F. arachnophila* is identical to *Aspergillus depauperatus*. Consequently the new combination is proposed:

*Filobasidiella depauperata* (Petch) comb. nov.

Basionym: *Aspergillus depauperatus* Petch – Trans. Br. Mycol. Soc. 16: 245, 1931.

Synonym: *Filobasidiella arachnophila* Malloch, Kane & Lahaie – Can. J. Bot. 56: 1823, 1978.

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